Autoxidation of Cod Liver Oil with Tocopherol and Ascorbyl Palmitate

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ABSTRACT: Cod liver oil (CLO) with no added antioxidants (REF), 200 mg/kg ascorbyl palmitate (AP), and/or 800 mg/kg tocopherol concentrate (TOH) were stored in sealed bottles with a small headspace of air at 25°C in the dark. A binary mix of TOH + AP affected the sensory perception of CLO by leading to a more grass/cucumber-like and less herring oil-like impression, whereas TOH alone had no effect. This was caused by the different influence of the antioxidants with regard to formation of volatile oxidation products. TOH + AP promoted formation of, e.g., hexanal, 2-hexenal, and 2,6-nonadienal and inhibited formation of, e.g., 2,4-heptadienal. TOH affected the proportions of *trans,cis*- and *trans,trans*-2,4-heptadienal that were formed and inhibited formation of, e.g., 1-penten-3-ol, whereas formation of acetic acid and some other volatiles was inhibited by both antioxidants. The total amount of volatiles increased during the experiment, and with REF were significantly higher ($P < 0.05$) than with TOH. The PV increased during the first 2 wk of storage. PV levels were in the order of TOH > REF > TOH + AP. The observed effects could partly be explained by hydrogen donation from TOH to peroxyl radicals, but the mode of action for AP was unclear.

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Cod liver oil (CLO) is a well-known nutritional supplement, traditionally consumed because of its content of vitamins A and D. However, it is also a good source of long-chained n-3 FA, mainly EPA and DHA. In the last decades, the increase in knowledge about the important health effects of these FA has created a renaissance for CLO.

It is well known that unsaturated FA are susceptible to oxidation. Their oxidizability is highly dependent on the number of doubly allylic hydrogen atoms present (1). The hydroperoxides that primarily are formed decompose to secondary oxidation products. Some of these are volatile compounds with low sensory thresholds and potentially great impact on the odor and flavor of the oil even in very low concentrations (1). The state-of-the-art knowledge about lipid oxidation reaction mechanisms, antioxidants, analytical methods, and other related aspects has been compiled (1). More recently, reviews on the role of hydroperoxides in lipid oxidation (2), kinetic evaluation of antioxidant activity (3), as well as inhibition of fish lipid oxidation with tocopherols (4) have been published.

Prevention of the oxidative deterioration of the odor and flavor of CLO and other highly unsaturated oils is not easily achieved, and although ternary blends of tocopherols, ascorbyl palmitate, and lecithin may effectively inhibit peroxidation, their ability to prevent formation of off-odors and -flavors is limited (5). Ranking of antioxidants in CLO is highly dependent on the methods used for evaluation, and the importance of using conditions relevant for normal storage and use has been pointed out (6). Antioxidants are usually multifunctional, and measurements of different types of oxidation products are necessary to avoid misleading interpretations of their effects (7). A consumer of CLO or other fish oil products will easily notice odor, flavor, and physical appearance of the oil, and these attributes are therefore important quality parameters. Sensory analysis must be regarded as the analytical technique giving information with the highest direct relevance for a consumer's perception of an oil and should be used whenever possible. Volatile oxidation products that may have an impact on odor and flavor can be analyzed by sensitive dynamic headspace/GC–MS techniques, whereas the PV gives information about primary oxidation products in the oil. Anisidine value (AnV) is one of the traditional methods for analysis of secondary oxidation products used in quality control, and measurement of induction time on a Rancimat instrument often has been applied in antioxidant evaluation.

The aim of this study was to evaluate the effects of ascorbyl palmitate (AP) and/or natural tocopherols (TOH) in autoxidizing CLO stored at conditions relevant for a real-life situation. The methods mentioned above were used to gain data about the oxidative status of the oil.

EXPERIMENTAL PROCEDURES

Sample preparation. Refined and deodorized CLO was from Peter Möller (Oslo, Norway). The CLO contained 0.1% FFA (8) and negligible trace amounts of α-tocopherol. The most abundant FA were C14:0 (3.4% of total FA), C16:0 (9.8%), C16:1n-7 (7.0%), C18:0 (2.2%), C18:1 (22.9%), C18:2n-6 (1.5%), C18:3n-3 (0.7%), C18:4n-3 (2.2%), C20:1 (13.1%),

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C20:5n-3 (9.5%), C22:1 (8.7%), C22:5n-3 (1.4%), and C22:6n-3 (13.3%). Tocopherol concentrate with >700 mg/g natural tocopherols (11% D-α, 61% D-β + D-γ, and 28% D-δ tocopherol) was purchased from Henkel (Düsseldorf, Germany). Ascorbyl palmitate and DL-α-tocopheryl acetate (food grade) were from Hoffman-La Roche (Basel, Switzerland).

Three batches of CLO were prepared: REF, control oil with no added antioxidants; TOH, 800 mg/kg tocopherol concentrate; and TOH + AP, 800 mg/kg tocopherol concentrate + 200 mg/kg ascorbyl palmitate. In addition to the antioxidants, 1600 mg/kg DL-α-tocopheryl acetate was added to all batches as customary for CLO. DL-α-Tocopheryl acetate provides vitamin E activity *in vivo* but has no antioxidant activity *in vitro* (9). The tocopherol concentrate was warmed to 40°C to lower the viscosity and then dissolved in CLO by stirring at room temperature for 20 min. Ascorbyl palmitate was dissolved in CLO as recommended by the manufacturer by first stirring it into CLO at 90–95°C. Then this premix was added to CLO at 60°C, stirred for 30 min, and cooled to room temperature before dilution in more CLO to the final AP concentration. The CLO was saturated with nitrogen prior to mixing, and all vials were flushed with nitrogen throughout the preparation procedure. Dark green, oval glass bottles were manually filled with 250 mL CLO and sealed immediately after preparation. The air headspace in the bottles was about 12 mL, and the surface area of the CLO was approximately 4.5 cm^2 (calculations based on bottle diameter and height). The bottles were placed at 25°C in a dark room with temperature control. Three randomly chosen bottles from each batch were collected as samples initially and after storage for 2, 5, 8, or 14 wk. The bottles were rotated by hand to mix the contents prior to opening, and the CLO from each bottle was split into smaller aliquots, flushed with argon (99.99%), and stored at –45°C until analysis.

Sensory analysis. A professional sensory panel with nine judges assessed the oil samples in a descriptive test according to an accredited method (ISO 6465:1985) (10). The panelists performed the analyses on 4-mL samples served in sealed vials at room temperature. The vials were labeled with random three-digit numbers and presented to the assessors in randomized order. All samples were served twice. The judges evaluated the intensity of odor and flavor of grass/cucumber, citrus, hay/dust, stearine/paraffin, paint, and herring oil, plus the intensity of bitter flavor. Scores were recorded on a linear scale from 1 (no intensity) to 9 (distinct intensity) using Compusense software (v. 5.38; Compusense Inc., Guelph, Canada).

PV and *AnV.* PV and AnV in the three sample types were analyzed initially and in samples stored for 2, 5, 8, and 14 wk with AOCS methods Cd 8-53 (11) and Cd 18-90 (12), respectively.

Volatile oxidation products. Volatile oxidation products were analyzed with a dynamic headspace/GC–MS method. An internal standard solution was prepared by stirring heptanoic acid ethyl ester (>99%; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) into soybean oil (Mills AS, Oslo, Norway) in a concentration of 52.6 μg/g. The fresh soybean oil contained only negligible traces of volatile oxidation products prior to use. CLO (5 g) and 0.1 g of the internal standard solution were accurately weighed into reagent tubes (50 mL) with glass stoppers. The samples were heated to 70°C for 10 min in a water bath and subsequently purged with 100 mL/min nitrogen through a modified glass tube for 20 min. Volatiles were collected on adsorbent tubes with a total of 1 mL Tenax GR (mesh size 60/80; Alltech Associates Inc., Deerfield, IL) and Carbosieve SIII (mesh size 60/80; Supelco, Bellefonte, PA), 1:1 vol/vol. Trapped compounds were desorbed in an automatic thermal desorption system and transferred to a gas chromatograph with a mass selective detector. The volatiles were separated on a DB-WAXetr column (30 m, 0.25 mm i.d., 0.5 μm film; J&W Scientific, Folsom, CA) with a temperature program starting at 30°C for 10 min, increasing 1°C/min to 40°C, 3°C/min to 70°C, and 6.5°C/min to 220°C, hold time 6 min. The components were tentatively identified with Wiley 130K Mass Spectral Database. The concentration of the individual volatiles in the samples was calculated by means of the internal standard and an external standard curve for hexanal. The latter was based on a solution of hexanal (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in soybean oil that was prepared the same way and with the same soybean oil as the internal standard solution. The external standard solution (in four different concentrations) was added to 5 g soybean oil together with 0.1 g internal standard solution and analyzed as regular samples. The ratio of the peak areas for hexanal and heptanoic acid ethyl ester were plotted as ordinate with the ratio of the respective amounts as abscissa, and the factor *a* from the resulting linear curve $y = ax + b$ was used as the response factor for hexanal. No corrections were made for the fact that other compounds with volatilities different from hexanal would yield other response factors, so the concentration of various volatiles was regarded as semiquantitative only.

Induction time. The induction time was measured with a Metrohm 679 Rancimat in which air (20 mL min^{-1}) was bubbled through 2.5 ± 0.05 g samples at 80°C. Changes in conductivity in 70 mL distilled water were recorded, and the induction time was calculated by the instrument.

Statistical analysis. The effects of the addition of TOH or TOH + AP compared with no antioxidants were evaluated at different storage times up to 14 wk. New, random bottles of CLO from the various treatments were chosen as samples at each storage time. ANOVA for the sensory results was performed with Statistix for Windows (v. 2.0; Analytical Software, Tallahassee, FL) with storage time (0, 2, 5, 8, and 14 wk), sample type (REF, TOH, and TOH + AP), and assessors as main effects. All interaction terms were included in the models. The assessor effect and all interactions involving the assessor effect were taken to be random effects, whereas the remaining effects were fixed. For the sensory attributes where the storage time or sample type effect (with assessor × storage and assessor × sample type as error terms, respectively) were significant at the 0.05 level, mean values were compared with Tukey's (HSD) Studentized test to decide which levels of storage times or sample types were significantly different.

For PV, AnV, volatile compounds, and induction time, ANOVA was performed with the General Linear Model

procedure in Minitab (v. 13.30; Minitab Inc., State College, PA) with storage time, sample type, and replicates as main effects. All two-factor interaction terms were included in the models. Effects involving the replicates were taken to be random. Tukey's test was performed to decide which levels of storage time or sample type were significantly different ($P < 0.05$) as described for the sensory data.

Partial least squares regression (PLSR) was used to generate regression models between sensory attributes and volatile components. All data were weighted to equal variance before analysis, and the models were cross-validated. The multivariate analysis was carried out with Unscrambler (v. 7.5; Camo A/S, Trondheim, Norway).

RESULTS AND DISCUSSION

Sensory analysis. The intensity of herring-oil flavor, paint flavor, and bitter flavor (Table 1) increased during the storage time, and the scores were significantly higher in week 14 compared with weeks 0, 2, and 5 ($P < 0.05$). Samples from week 8 also showed significantly higher intensity of these attributes compared with samples from week 0, but were not different from weeks 2 and 5 ($P < 0.05$). This indicated a relatively slow development of sensory attributes that might be associated with rancidity in the oil. CLO with TOH + AP received significantly lower sensory scores for herring-oil flavor than the reference batch ($P < 0.05$). Paint flavor seemed to develop the same way as herring-oil flavor, but the differences between the treatments were not significant ($P = 0.061$) for this attribute. Grass/cucumber flavor developed along a slightly different pattern (Table 1). The intensity of this attribute was significantly higher in weeks 2 and 14 than in week 0 ($P < 0.05$), and TOH + AP received significantly higher scores than TOH ($P < 0.05$). Stearine/paraffin flavor (Table 1) increased significantly ($P < 0.05$) during the first 2 wk of the storage time, whereas other flavors remained stable throughout the experiment. The odor attributes generally developed along the same trends as the corresponding flavors (Table 1).

Previous attempts to protect fish oils against the development of an unpleasant fishy odor and flavor have not been particularly successful (5). Karahadian and Lindsay (13) observed a progression from green and cucumber to fishy and rancid notes in fish oil oxidizing under fluorescent light. In the present study, addition of a binary mix of TOC and AP affected the sensory perception of the CLO by leading to a more grass/cucumber-like and less herring oil-like impression. Although TOH + AP did not completely inhibit formation of off-odors and -flavors in the CLO, the data suggested that TOH + AP could have a practical impact. The data from Karahadian and Lindsay (13) suggest that herring oil-like notes could be perceived as more oxidized than green/grassy notes; and if grass/cucumber odor and flavor would be regarded as more acceptable than herring oil flavor or paint flavor, addition of TOH + AP could be useful. However, these results were gained from a trained sensory panel, and no assumptions could be made as to whether ordinary consumers would notice the differences between the various treatments.

PV. The PV increased significantly ($P < 0.05$) during the first 2 wk of storage (Table 2). There was a small significant decrease from week 2 to week 8 ($P < 0.05$), but no change from then on to week 14. The three treatments were significantly different ($P < 0.05$), with decreasing PV levels in the order of TOH > REF > TOH + AP.

No analyses were performed during the first 2 wk of storage, so there was no way to tell how early the PV actually started to increase. However, considering the high abundance of EPA and DHA in the oil, the access to oxygen in the bottle

TABLE 1
Sensory Attributes in Cod Liver Oil (CLO) During Storage at 25°C for 14 wk[a]

Storage time (wk)	REF (score[b])					TOH (score[b])					TOH + AP (score[b])				
	0	2	5	8	14	0	2	5	8	14	0	2	5	8	14
Odors															
Grass/cucumber	$2.7^{a,x}$	$3.5^{b,x}$	$3.0^{a,b,x}$	$3.3^{b,x}$	$3.2^{a,b,x}$	$2.3^{a,x}$	$3.2^{b,x}$	$3.3^{a,b,x}$	$3.1^{b,x}$	$3.2^{a,b,x}$	$3.2^{a,y}$	$3.7^{b,y}$	$3.7^{a,b,y}$	$4.2^{b,y}$	$3.4^{a,b,y}$
Citrus	2.0	1.7	1.8	1.7	2.0	1.5	1.6	1.8	1.8	1.6	2.3	1.8	2.2	2.5	2.2
Stearine/paraffin	2.0^{a}	$2.7^{a,b}$	$2.5^{a,b}$	2.7^{b}	2.9^{b}	2.1^{a}	$2.7^{a,b}$	$2.5^{a,b}$	2.9^{b}	2.9^{b}	2.1^{a}	$2.3^{a,b}$	$2.3^{a,b}$	2.3^{b}	2.6^{b}
Herring oil	1.3^{a}	$1.9^{a,b}$	$2.3^{a,b,c}$	$2.7^{b,c}$	2.6^{c}	1.4^{a}	$2.0^{a,b}$	$1.9^{a,b,c}$	$2.4^{b,c}$	2.9^{c}	1.6^{a}	$1.6^{a,b}$	$1.8^{a,b,c}$	$1.9^{b,c}$	2.4^{c}
Hay/dust	1.4	1.4	1.5	1.8	1.6	1.4	1.5	1.5	1.7	1.6	1.3	1.6	1.4	1.7	1.4
Paint	1.1^{a}	$1.6^{a,b}$	$1.6^{a,b}$	1.9^{b}	2.5^{c}	1.0^{a}	$1.7^{a,b}$	$1.2^{a,b}$	1.9^{b}	2.6^{c}	1.4^{a}	$1.4^{a,b}$	$1.3^{a,b}$	1.3^{b}	1.9^{c}
Flavors															
Grass/cucumber	$3.0^{a,x,y}$	$3.6^{b,x,y}$	$3.2^{a,b,x,y}$	$3.1^{a,b,x,y}$	$3.4^{b,x,y}$	$2.3^{a,x}$	$3.6^{b,x}$	$3.4^{a,b,x}$	$3.2^{a,b,x}$	$3.4^{b,x}$	$3.2^{a,y}$	$3.8^{b,y}$	$3.7^{a,b,y}$	$4.3^{a,b,y}$	$4.1^{b,y}$
Citrus	1.8	1.7	1.9	1.6	1.5	1.6	1.7	1.8	1.7	1.7	2.2	1.8	2.3	2.5	2.2
Stearine/paraffin	2.4^{a}	3.0^{b}	$3.0^{a,b}$	3.0^{b}	3.2^{b}	2.2^{a}	2.9^{b}	$2.9^{a,b}$	2.9^{b}	3.1^{b}	2.5^{a}	2.6^{b}	$2.5^{a,b}$	2.9^{b}	3.1^{b}
Herring oil	$1.5^{a,y}$	$2.5^{b,y}$	$3.0^{b,y}$	$3.3^{b,c,y}$	$3.8^{c,y}$	$1.3^{a,x,y}$	$2.6^{b,x,y}$	$2.4^{b,x,y}$	$3.0^{b,c,x,y}$	$3.6^{c,x,y}$	$1.5^{a,x}$	$1.9^{b,x}$	$2.3^{b,x}$	$2.4^{b,c,x}$	$2.7^{c,x}$
Hay/dust	1.3	1.5	1.7	1.8	1.8	1.3	1.6	1.6	1.8	1.7	1.5	1.5	1.4	1.7	1.7
Paint	1.1^{a}	$2.2^{a,b}$	2.2^{b}	$2.6^{b,c}$	3.3^{c}	1.1^{a}	$2.0^{a,b}$	1.9^{b}	$2.5^{b,c}$	3.1^{c}	1.3^{a}	$1.4^{a,b}$	1.5^{b}	$1.8^{b,c}$	2.4^{c}
Bitter	1.9^{a}	$2.4^{a,b}$	2.5^{b}	$2.9^{b,c}$	3.1^{c}	1.9^{a}	$2.4^{a,b}$	2.4^{b}	$2.7^{b,c}$	3.0^{c}	2.0^{a}	$2.2^{a,b}$	2.5^{b}	$2.6^{b,c}$	2.8^{c}

[a]REF, control oil with no added antioxidants; TOH, 800 mg/kg tocopherol concentrate; and TOH + AP, 800 mg/kg tocopherol concentrate + 200 mg/kg ascorbyl palmitate (AP).

[b]Averaged sensory scores from nine panelists assessing the samples twice. Values within a row with unlike superscripts (a–d) indicate significant differences (Tukey's test, $P < 0.05$) between storage times (0, 2, 5, 8, and 14 wk). Values within a row with unlike superscripts (x–z) indicate significant differences (Tukey's test, $P < 0.05$) between sample types (i.e., REF, TOH, and TOH + AP).

TABLE 2
PV in Cod Liver Oil (CLO) During Storage at 25°C for 14 wk[a]

Storage time (wk)	REF[b]					TOH[b]					TOH + AP[b]				
	0	2	5	8	14	0	2	5	8	14	0	2	5	8	14
PV (mequiv/kg)	$0.8^{a,x}$	$3.1^{b,x}$	$2.4^{b,c,x}$	$2.4^{d,x}$	$2.5^{c,d,x}$	$0.8^{a,y}$	$3.3^{b,y}$	$3.1^{b,c,y}$	$2.8^{d,y}$	$2.8^{c,d,y}$	$0.6^{a,z}$	$2.5^{b,z}$	$2.7^{b,c,z}$	$2.2^{d,z}$	$2.3^{c,d,z}$

[a]Averaged values, $n = 3$.
[b]Values with unlike superscripts (a–c) indicate significant differences (Tukey's test, $P < 0.05$) between storage times (0, 2, 5, 8, and 14 wk). Values with unlike superscripts (x–z) indicate significant differences (Tukey's test, $P < 0.05$) between sample types (i.e., REF, TOH, and TOH + AP). For abbreviations see Table 1.

headspace, and the storage temperature at 25°C, one would expect either immediate production of hydroperoxides or a very short lag time. This would be in accordance with data for oxidation of menhaden oil stored at 30°C in the dark (4). Lipid oxidation proceeds independently of oxygen pressure when oxygen is freely available, but if the oxygen availability is restricted, the oxidation rate becomes oxygen dependent (1). Oxygen consumption could not be measured in the bottles that were used. However, based on the initial increase and subsequent small decrease in PV, it would not be unreasonable to think that restricted oxygen availability was a limiting factor for formation of oxidation products during the later stages of this experiment.

Volatile oxidation products. The most abundant FA in CLO are saturated or monounsaturated, or they contain five or six double bonds (EPA and DHA). The oxidizability of FA is highly dependent on the number of doubly allylic positions available (1). As a result, EPA and DHA are expected to be precursors of the major oxidation products found in CLO. Several papers with analyses of volatile oxidation products in fish oils have been published (e.g., 13–17). The data vary from study to study owing to differences in sampling and chromatographic techniques as well as varying storage conditions and oxidation levels. The volatile components identified in the present CLO samples were mainly in good accordance with compounds previously reported in other studies (13–17).

The total amount of volatiles increased during the experiment (Table 3). All storage times were significantly different ($P < 0.05$) except weeks 2 and 5. REF had a significantly higher total level of volatiles than TOH ($P < 0.05$). The individual volatile oxidation products that increased during the storage time are shown in Table 3. Several other components that either remained stable for 14 wk or were found in trace amounts in a few of the samples were detected (not shown).

The concentrations of the various volatiles that were formed differed in the three treatments. For some of the components no special pattern could be seen, but others appeared to develop along four main trends for which different effects of the antioxidants could be observed.

(i) Promotion by TOH, inhibition by TOH + AP. Formation of one group of components was significantly promoted by TOH but inhibited by TOH + AP ($P < 0.05$), as seen for *trans,cis*-2,4-heptadienal (Table 3). Other components following this pattern were, e.g., a 2,5-octadiene isomer and 1,6-hexadiene (Table 3).

(ii) Inhibition by TOH and TOH + AP. TOH and TOH + AP significantly inhibited ($P < 0.05$) formation of some components that increased rapidly during the first part of the storage time before the formation rate seemed to diminish. 1-Penten-3-ol and 2-propenal (Table 3) were examples of such compounds.

(iii) Inhibition by TOH + AP more effective than by TOH alone. Formation of a third group of components was significantly inhibited by both antioxidant treatments, and TOH + AP was more effective that TOH alone ($P < 0.05$). This was observed for, e.g., acetic acid, propionic acid, 2-ethyl furan, and 2,3-pentanedione (Table 3). These compounds increased steadily throughout the storage time.

(iv) Promotion by TOH + AP, lesser effect from REF and TOH alone. A fourth group of components, e.g., hexanal (Table 3), was mainly formed in samples containing TOH + AP and only to a lesser extent in the REF and TOH samples. The differences were significant ($P < 0.05$). This trend was also apparent for 2-hexenal, 2,6-nonadienal, and propanal (Table 3). The level of these components was higher in samples with TOH + AP right from the start, indicating that some of them initially formed during the process of solubilizing the AP in the CLO. This corresponded to a slightly lower PV in TOH + AP than in the other samples when the experiment started.

It is well known that antioxidants may have an effect on the formation and decomposition of lipid hydroperoxides. The patterns for formation of volatile oxidation products found in this study illustrated different types of antioxidative activity. *trans,cis*- and *trans,trans*-2,4-Heptadienal (Table 3) showed a fairly steady increase in REF samples during the storage time. Compared with this, TOH promoted formation of *trans,cis*- and inhibited formation of *trans*,*trans*-2,4-heptadienal. The sums of the two heptadienal isomers in REF and TOH were equal.

Probably the most recognized antioxidative effect of tocopherols is their ability to donate hydrogen atoms to peroxyl radicals. *cis,trans*-Hydroperoxides can isomerize to *trans,trans*-hydroperoxides *via* peroxyl radicals, and tocopherols may inhibit this reaction to different extents depending on their hydrogen-donating power and concentration. Kulås *et al.* (14) suggested that the subsequent decomposition of hydroperoxides *via* alkoxyl radicals could give an increased ratio of *trans*,*cis*- to *trans*,*trans*-2,4-heptadienal. The present data for CLO were in accordance with this, with a *trans,cis*- to *trans,trans*- ratio of 3:2 in the REF compared with 3:1 in TOH after 14 wk of storage. The tocopherol mix used in this study

TABLE 3
Volatile Compounds in Cod Liver Oil (CLO) During Storage at 25°C for 14 wk

RT (min)	Storage time (wk)	REF (ng/g) 0	REF (ng/g) 2	REF (ng/g) 5	REF (ng/g) 8	REF (ng/g) 14	TOH (ng/g) 0	TOH (ng/g) 2	TOH (ng/g) 5	TOH (ng/g) 8	TOH (ng/g) 14	TOH + AP (ng/g) 0	TOH + AP (ng/g) 2	TOH + AP (ng/g) 5	TOH + AP (ng/g) 8	TOH + AP (ng/g) 14
3.4	Acetaldehyde	$34.9^{a,x}$	$86.7^{b,x}$	$81.0^{b,x}$	$113.6^{b,x}$	$145.1^{c,x}$	$36.5^{a,x,y}$	$80.0^{b,x,y}$	$65.9^{b,x,y}$	$80.4^{b,x,y}$	$138.4^{c,x,y}$	$32.4^{a,y}$	$53.3^{b,y}$	$66.2^{b,y}$	$64.8^{b,y}$	$113.5^{c,y}$
4.8	Propanal	$24.9^{a,x}$	$65.0^{a,b,x}$	$63.9^{b,x}$	$94.1^{b,x}$	$113.3^{c,x}$	$22.3^{a,x}$	$45.7^{a,b,x}$	$48.1^{b,x}$	$53.7^{b,x}$	$95.3^{c,x}$	$59.3^{a,y}$	$71.9^{a,b,y}$	$101.3^{b,y}$	$90.8^{b,y}$	$261.8^{c,y}$
6.5	2-Propenal	$46.2^{a,x}$	$152.0^{b,x}$	$116.5^{b,x}$	$137.2^{b,x}$	$123.9^{b,x}$	$48.7^{a,y}$	$108.2^{b,y}$	$83.5^{b,y}$	$92.0^{b,y}$	$106.2^{b,y}$	$36.6^{a,z}$	$67.7^{b,z}$	$85.5^{b,z}$	$82.1^{b,z}$	$91.1^{b,z}$
8.0	1,6-Hexadiene		$15.1^{a,x}$	$22.7^{b,x}$	$36.9^{c,x}$	$60.1^{d,x}$		$19.7^{a,y}$	$29.1^{b,y}$	$54.2^{c,y}$	$85.5^{d,y}$		$13.5^{a,z}$	$22.3^{b,z}$	$26.4^{c,z}$	$48.9^{d,,z}$
9.6	2,5-Octadiene		$37.6^{a,x}$	$74.9^{b,x}$	$111.7^{c,x}$	$175.3^{d,x}$		$49.9^{a,y}$	$91.0^{b,y}$	$165.6^{c,y}$	$291.5^{d,y}$		$32.7^{a,z}$	$69.4^{b,z}$	$81.2^{c,z}$	$148.0^{d,z}$
10.4	2,5-Octadiene		$14.0^{a,b,x}$	$30.0^{a,c,x}$	$41.5^{a,d,x}$	$73.6^{e,x}$		$20.6^{a,b,y}$	$40.1^{a,c,y}$	$68.5^{a,d,y}$	$123.5^{e,y}$	$8.5^{a,x}$	$14.3^{a,b,x}$	$27.1^{a,c,x}$	$33.9^{a,d,x}$	$61.0^{e,x}$
11.3	Ethanol	78.4^{a}	92.2^{a}	$71.4^{a,b}$	$89.4^{a,b,c}$	$116.9^{a,c}$	80.9^{a}	114.9^{a}	$59.4^{a,b}$	$71.1^{a,b,c}$	$88.7^{a,b,c}$	58.3^{a}	128.3^{a}	$82.1^{a,b}$	$79.2^{a,b,c}$	$84.8^{a,b,c}$
12.5	2-Ethyl furan	$26.8^{a,x}$	$51.7^{b,x}$	$61.7^{c,x}$	$71.4^{d,x}$	$85.6^{e,x}$	$23.6^{a,y}$	$38.6^{b,y}$	$45.3^{c,y}$	$51.1^{d,y}$	$65.2^{e,y}$	$10.9^{a,z}$	$30.9^{b,z}$	$38.5^{c,z}$	$32.9^{d,z}$	$46.3^{e,z}$
14.4	Pentanal	$41.7^{a,x}$	$79.7^{b,x}$	$90.0^{c,x}$	$110.8^{c,x}$	$139.4^{d,x}$	$58.3^{a,x}$	$72.1^{b,x}$	$73.1^{c,x}$	$87.8^{c,x}$	$141.7^{d,x}$	$50.4^{a,y}$	$98.2^{b,y}$	$127.3^{c,y}$	$119.5^{c,y}$	$199.9^{d,y}$
15.1	2,3-Butanedione	$14.7^{a,x}$	$31.1^{b,x}$	$43.8^{b,x}$	$50.6^{b,x}$	$43.6^{b,x}$	$24.0^{a,x}$	$32.2^{b,x}$	$38.7^{b,x}$	$33.0^{b,x}$	$26.6^{b,x}$	$13.6^{a,y}$	$15.0^{b,y}$	$22.6^{b,y}$	$17.5^{b,y}$	$21.9^{b,y}$
18.4	1-Penten-3-one		$60.0^{b,x}$	$42.4^{b,x}$	$54.6^{b,c,x}$	$55.8^{c,x}$		$39.5^{b,y}$	$35.7^{b,y}$	$42.2^{b,c,y}$	$55.9^{c,y}$	$15.0^{a,y}$	$38.6^{b,y}$	$46.2^{b,y}$	$44.9^{b,c,y}$	$47.9^{c,y}$
20.4	2-Butenal (*E*)		$24.1^{a,x}$	$24.2^{a,x}$	$24.1^{a,x}$	$34.3^{b,x}$		$13.5^{a,y}$	$14.3^{a,y}$	$16.9^{a,y}$	$29.8^{b,y}$		$17.2^{a,x}$	$20.6^{a,x}$	$19.0^{a,x}$	$42.8^{b,x}$
23.1	2,3-Pentanedione		$33.4^{a,x}$	$49.7^{b,x}$	$62.3^{c,x}$	$71.7^{d,x}$		$27.7^{a,x}$	$38.8^{b,x}$	$48.1^{c,x}$	$66.3^{d,x}$		$9.8^{a,x}$	$17.4^{b,x}$	$19.3^{c,x}$	$43.2^{d,x}$
24.4	Hexanal	$21.4^{a,x}$	$44.6^{b,x}$	$41.8^{b,x}$	$49.1^{b,x}$	$65.2^{c,x}$	$19.9^{a,x}$	$30.2^{b,x}$	$29.5^{b,x}$	$31.4^{b,x}$	$85.8^{c,x}$	$56.4^{a,y}$	$81.0^{b,y}$	$111.4^{b,y}$	$112.2^{b,y}$	$193.4^{c,y}$
25.6	1,3,6-Octatriene		$13.3^{a,x}$	$16.2^{b,x}$	$20.5^{b,x}$	$29.3^{c,x}$		$9.8^{a,y}$	$14.7^{b,y}$	$16.9^{b,y}$	$24.9^{c,y}$		$9.1^{a,x}$	$18.2^{b,x}$	$18.0^{b,x}$	$30.8^{c,x}$
26.6	2-Pentenal (*Z*)	$27.6^{a,x}$	$35.3^{a,b,x}$	$26.7^{a,x}$	$34.8^{a,b,x}$		$18.4^{a,y}$	$27.2^{a,b,y}$	$22.5^{a,y}$	$31.4^{a,b,y}$		$29.7^{a,x,y}$	$33.9^{a,b,x,y}$	$18.7^{a,x,y}$	$24.3^{a,b,x,y}$	
28.3	2-Pentenal (*E*)	$15.3^{a,x}$	$33.5^{b,x}$	$38.6^{b,c,x}$	$47.4^{c,x}$	$64.9^{d,x}$	$16.2^{a,y}$	$21.9^{b,y}$	$27.5^{b,c,y}$	$34.7^{c,y}$	$59.9^{d,y}$	$12.9^{a,x}$	$41.0^{b,x}$	$46.2^{b,c,x}$	$41.3^{c,x}$	$65.3^{d,x}$
31.4	1-Penten-3-ol	$67.0^{a,x}$	$302.9^{b,x}$	$233.2^{b,x}$	$246.6^{b,x}$	$242.3^{b,x}$	$61.7^{a,y}$	$178.1^{b,y}$	$153.2^{b,y}$	$166.6^{b,y}$	$214.6^{b,y}$	$46.0^{a,y}$	$142.1^{b,y}$	$166.1^{b,y}$	$145.6^{b,y}$	$182.8^{b,y}$
31.9	Heptanal		$30.2^{a,x,y}$	$26.7^{a,b,x,y}$	$33.1^{a,b,c,x,y}$	$49.7^{a,c,x,y}$		$31.4^{a,x}$	$27.3^{a,b,x}$	$25.2^{a,b,c,x}$	$32.7^{a,c,x}$		$32.8^{a,y}$	$34.0^{a,b,y}$	$42.4^{a,b,c,y}$	$49.5^{a,c,y}$
33.1	2-Hexenal (*Z*)			$5.5^{a,x}$		$10.5^{b,x}$			$3.6^{a,x}$		$12.6^{b,x}$		$11.7^{a,y}$	$19.6^{a,y}$	$20.5^{a,y}$	$43.9^{b,y}$
33.8	2-Hexenal (*E*)			$17.2^{a,x}$	$12.4^{a,x}$	$43.0^{b,x}$			$18.8^{a,x}$	$11.2^{a,x}$	$54.6^{b,x}$		$73.1^{a,y}$	$120.1^{a,y}$	$137.5^{a,y}$	$258.4^{b,y}$
35.5	1-Pentanol		11.1^{a}	10.7^{a}	26.9^{a}	69.7^{b}		13.4^{a}	21.9^{a}	36.1^{a}	70.2^{b}		8.7^{a}	30.8^{a}	24.0^{a}	51.9^{b}
36.6	Octanal	$12.2^{a,x}$	$19.5^{b,x}$	$13.3^{a,b,c,x}$	$16.9^{b,x}$	$33.2^{b,c,x}$		$20.2^{b,x}$	$12.7^{a,b,c,x}$	$13.4^{b,x}$	$17.6^{b,c,x}$	$9.1^{a,y}$	$22.3^{b,y}$	$25.6^{a,b,c,y}$	$31.3^{b,y}$	$41.3^{b,c,y}$
37.1	4-Heptenal/ 2-heptenal (*Z*)	10.8^{a}	12.1^{a}	15.9^{a}	16.5^{a}	42.0^{b}		9.0^{a}	13.2^{a}	12.8^{a}	70.8^{b}		21.0^{a}	12.8^{a}	11.6^{a}	30.4^{b}
40.0	Nonanal	17.2^{a}	33.9^{b}	$25.4^{a,b}$	38.6^{b}	68.6^{b}	14.6^{a}	42.8^{b}	$23.0^{a,b}$	27.7^{b}	28.5^{b}	14.0^{a}	34.5^{b}	$24.7^{a,b}$	42.0^{b}	47.2^{b}
41.1	2-Octenal (*E*)	10.4^{a}	$13.8^{a,b}$	$10.8^{a,b,c}$	$14.3^{a,b,c}$	28.9^{b}	8.7^{a}	$15.4^{a,b}$	$9.3^{a,b,c}$	$10.2^{a,b,c}$	16.5^{b}	6.6^{a}	$14.5^{a,b}$	$11.8^{a,b,c}$	$12.5^{a,b,c}$	19.7^{b}
41.8	Acetic acid	$77.1^{a,x}$	$275.4^{b,x}$	$440.2^{c,x}$	$655.2^{d,x}$	$836.9^{e,x}$	$82.1^{a,y}$	$247.1^{b,y}$	$383.5^{c,y}$	$561.2^{d,y}$	$671.7^{e,y}$	$91.8^{a,z}$	$189.0^{b,z}$	$249.7^{c,z}$	$239.4^{d,z}$	$421.5^{e,z}$
42.1	2,4-Heptadienal (*E,Z*)	$28.4^{a,x}$	$59.0^{b,x}$	$83.8^{c,x}$	$114.1^{d,x}$	$145.5^{e,x}$	$32.6^{a,y}$	$60.1^{b,y}$	$92.5^{c,y}$	$133.6^{d,y}$	$182.0^{e,y}$	$22.9^{a,z}$	$39.7^{b,z}$	$73.6^{c,z}$	$68.1^{d,z}$	$86.7^{e,z}$
42.8	2,4-Heptadienal (*E,E*)	27.7^{a}	$36.7^{a,b}$	$45.8^{a,b,c}$	$63.5^{b,c}$	98.0^{b}	31.4^{a}	$37.5^{a,b}$	$42.5^{a,b,c}$	$54.8^{b,c}$	63.2^{b}	25.3^{a}		$49.0^{a,b,c}$	$50.9^{b,c}$	51.9^{b}
43.7	Benzaldehyde/ 2-nonenal	22.3^{a}	34.6^{b}	38.3^{b}	$36.3^{a,b}$	58.0^{b}	27.3^{a}	37.2^{b}	32.8^{b}	$28.0^{a,b}$	36.2^{b}		27.7^{b}	40.8^{b}	$33.7^{a,b}$	37.2^{b}
43.8	Propanoic acid	$16.1^{a,x}$	$52.7^{b,x}$	$86.4^{c,x}$	$119.7^{c,x}$	$195.0^{d,x}$	$20.1^{a,x}$	$41.2^{b,x}$	$71.6^{c,x}$	$103.8^{c,x}$	$172.1^{d,x}$	$19.6^{a,y}$	$32.7^{b,y}$	$45.3^{c,y}$	$42.0^{c,y}$	$92.3^{d,y}$
44.9	2,6-Nonadienal	$6.5^{a,x}$	$9.2^{a,x}$	$5.7^{a,x}$	$9.2^{a,x}$	$42.0^{b,x}$	$6.4^{a,x}$		$5.6^{a,x}$	$8.7^{a,x}$	$15.0^{b,x}$	$5.6^{a,y}$	$15.8^{a,y}$	$24.2^{a,y}$	$35.1^{a,y}$	$61.8^{b,y}$
45.1	Dodecanal	10.2^{a}	27.8^{b}	15.9^{a}	$25.4^{a,b}$	18.7^{b}	6.4^{a}	29.0^{b}	18.4^{a}	$17.9^{a,b}$	25.7^{b}	8.0^{a}	29.2^{b}	19.7^{a}	$21.6^{a,b}$	48.9^{b}
45.7	Butyric acid		$11.2^{a,x}$	$15.5^{b,x}$	$16.1^{b,x}$	$22.8^{c,x}$		$9.9^{a,y}$	$14.4^{b,y}$	$13.9^{b,y}$	$16.9^{c,y}$		$7.6^{a,x,y}$	$12.2^{b,x,y}$	$11.2^{b,x,y}$	$26.5^{c,x,y}$
46.1	2-Decenal	26.4^{a}	40.6^{b}	$26.2^{a,b}$	$37.6^{a,b}$	101.0^{b}	25.8^{a}	49.0^{b}	$24.1^{a,b}$	$28.5^{a,b}$	45.7^{b}	16.5^{a}	40.1^{b}	$36.9^{a,b}$	$40.1^{a,b}$	61.2^{b}
47.3	Cycloocta-1,5-dien-7-one	12.6^{a}	13.8^{a}	$20.6^{a,b}$	$20.6^{a,b}$	29.9^{b}	12.7^{a}	16.7^{a}	$16.0^{a,b}$	$17.2^{a,b}$	23.2^{b}	8.0^{a}	14.6^{a}	$19.4^{a,b}$	$19.3^{a,b}$	23.7^{b}
48.2	2-Undecenal	18.4^{a}	55.1^{b}	$27.2^{a,b}$	$41.5^{a,b}$	83.8^{b}	18.0^{a}	51.1^{b}	$27.1^{a,b}$	$30.9^{a,b}$	33.8^{b}	12.8^{a}	37.2^{b}	$29.6^{a,b}$	$38.5^{a,b}$	53.3^{b}
48.4	2,4-Decadienal (*Z,E*)	10.2^{a}	$14.9^{a,b}$	17.6^{a}	$24.2^{a,c}$	$34.7^{b,c}$	12.6^{a}	$15.7^{a,b}$	14.9^{a}	$18.4^{a,c}$	$27.7^{b,c}$		$13.6^{a,b}$	18.1^{a}	$20.1^{a,c}$	$24.8^{b,c}$
49.5	2,4-Decadienal (*E,E*)	13.7^{a}	$9.9^{a,b}$	$11.0^{a,b,c}$	$17.0^{a,b,c}$	24.6^{b}	11.1^{a}	$10.9^{a,b}$	$9.5^{a,b,c}$	$15.7^{a,b,c}$	21.5^{b}	5.8^{a}	$13.3^{a,b}$	$11.9^{a,b,c}$	$12.4^{a,b,c}$	19.8^{b}
49.9	Hexanoic acid	12.8^{a}	$19.9^{a,b}$	$21.2^{a,b}$	22.8^{b}	27.2^{b}	9.4^{a}	$25.4^{a,b}$	$16.5^{a,b}$	18.5^{b}	21.7^{b}	8.8^{a}	$15.2^{a,b}$	$19.1^{a,b}$	24.5^{b}	28.8^{b}
52.2	1-Ethoxy-4-methyl-2-pentene	28.0^{a}	50.8^{b}	$43.8^{a,b}$	57.0^{b}	49.8^{b}	23.1^{a}	42.5^{b}	$41.1^{a,b}$	43.0^{b}	55.6^{b}	15.2^{a}	28.0^{b}	$38.4^{a,b}$	38.8^{b}	57.0^{b}
	Total volatiles	$1289^{a,x}$	$2718^{b,x}$	$2917^{b,x}$	$3785^{c,x}$	$4767^{d,x}$	$1072^{a,y}$	$2426^{b,y}$	$2607^{b,y}$	$3285^{c,y}$	$4428^{d,y}$	$1314^{a,x,y}$	$2415^{b,x,y}$	$2984^{b,x,y}$	$3056^{c,x,y}$	$4689^{d,x,y}$

[a]Averaged values, $n = 3$.

[b]Values within a row with unlike superscripts (a–c) are significantly different (Tukey's test, $P < 0.05$) with regard to storage time. Values within a row with unlike superscripts (x–z) indicate significant differences (Tukey's test, $P < 0.05$) between sample types (i.e., REF, TOH, and TOH + AP). For abbreviations see Table 1.

therefore had enough hydrogen-donating power to partially inhibit isomerization, even though the content of α-tocopherol was low. The binary antioxidant mix had a different impact on formation of the two 2,4-heptadienal isomers than TOH alone, and this was attributed to the effect of AP. TOH + AP inhibited formation of both 2,4-heptadienals but apparently not the *cis,trans*- to *trans,trans*- isomerization. This led to the assumption that AP had little or no effect with regard to scavenging of peroxyl radicals in CLO.

TOH and TOH + AP inhibited formation of 1-penten-3-ol (Table 3) the same way, so AP did not have an effect on the mechanism for the formation of this component. 1-Penten-3-ol can be a tertiary oxidation product from further oxidation of 2,4-heptadienal. Hydrogen donors such as tocopherols could protect unsaturated aldehydes from oxidative degradation, leading to lower levels of 1-penten-3-ol (14). The formation of 1-penten-3-ol and the other volatile oxidation products in the second group above seemed more or less to follow the pattern of the PV (Table 2) with the largest increase during the first 2 wk of storage, whereas the compounds in the other groups increased throughout the storage time. This could indicate that formation of the oxidation products in the second group were more dependent on oxygen pressure than formation of some of the other components.

TOH + AP inhibited formation of acetic acid (Table 3) more effectively than TOH alone. Acids could be tertiary oxidation products formed from the corresponding primary aldehydes (1), and tocopherols might protect aldehydes from further oxidation. From the present data, it was not possible to say whether the higher inhibitory effect of TOH + AP was caused by a specific antioxidative effect of AP or whether it was a synergistic effect of tocopherols and AP. AP can reduce tocopheroxyl radicals back to the original tocopherol, so that the tocopherols are spared and a synergistic effect is observed (1).

TOH had no influence on the formation of hexanal (Table 3) and the other components listed in the fourth group listed above, whereas TOH + AP increased the formation of these components. This effect must have been caused by the AP. Mäkinen *et al*. (18) found that AP slightly increased the decomposition rate of methyl linoleate hydroperoxides owing to its reductive activity on metal ions. They stated that under favorable circumstances AP could act as a hydrogen atom donor to peroxyl radicals or reduce hydroperoxides to more stable hydroxy compounds, but because these effects were small, they thought that AP mainly worked because of synergistic interactions with other antioxidants. As already discussed, AP did not appear to scavenge peroxyl radicals in CLO, and no conclusion regarding its activity toward reduction of hydroperoxides could be made. The PV level in samples with TOH + AP were slightly lower than in the two other treatments, although the total level of volatiles was the same as in the REF, so AP might have increased the hydroperoxy decomposition rate marginally. However, the main effect of AP in the CLO appeared to be quite specific interactions with one or more scission pathways.

The grouping of volatiles as just described was apparent when the development of single components was followed throughout the storage time. The connection between the different types of volatiles and the sensory attributes could be illustrated by multivariate analysis of the data. In a PLS2 regression model with all the volatiles and grass/cucumber, herring oil, paint, and bitter flavors, correlation factors for prediction of the sensory parameters of 0.59, 0.92, 0.95, and 0.93, respectively, were found. It was apparent that herring oil, paint, and bitter flavors co-varied with each other and with a major part of the volatiles, e.g., 2,4-heptadienal, 2,5-octadiene, 2-ethylfuran, 1-penten-3-ol, 2,3-pentanedione, and acetic acid. Grass/cucumber flavor co-varied with hexanal, 2,6-nonadienal, propanal, 2-hexenal, and a few other volatiles. Hexanal has a pungent, green, and grassy odor (17), and 2,6-nonadienal has a cucumber odor (17). The odor of propanal is pungent and penetrating (19), whereas 2-hexenal is green and leafy (19). It seems highly likely that higher levels of these compounds combined with lower levels of some other components could lead to a more grass/cucumber-like impression of the CLO samples with TOH + AP. The regression model worked less well for grass/cucumber flavor than for the other sensory attributes. This could be due to the influence of trace amounts of components having very low threshold values and a potentially "green" impact on the sensory data. Herring oil, paint, and bitter flavors, but not grass/cucumber flavor could be equally well predicted by a model with only 2,3-pentandione, *t,c*-2,4-heptadienal, acetic acid, and propionic acid as with the model with all the volatile compounds.

AnV. The AnV remained stable at 14 for all treatments throughout the storage time (data not shown). In the AOCS method (12), the AnV is attributed to the amount of unsaturated aldehydes with double bonds in the 2- or 2,4-positions. In the present study, several volatiles corresponding to the former description (e.g., 2-propenal, 2-hexenal, and 2,4-heptadienal) increased significantly during the storage time. However, AnV was not sensitive enough to reflect these changes, and the values were regarded as mainly representing the level of core aldehydes in the oil after processing.

Induction time. The REF, TOH, and TOH + AP samples were significantly different ($P < 0.05$), with initial induction times of 4.3, 8.8, and 11.9 h, respectively. (Corresponding SD were 0.27, 0.06, and 0.21 h.) No changes appeared during storage. In considering the increase in volatile compounds as well as the flavor deterioration, the induction time did not give any useful information about the resistance of the oils toward autoxidation at 25°C.

Sensory analysis indicated that TOH + AP had an effect on grass/cucumber and herring-oil flavors. This could be interesting information with regard to oil quality and effect of antioxidants from a consumer-oriented perspective. The PV data were not very informative on their own but could be useful in connection with the measurement of volatiles. Dynamic headspace/GC–MS analysis showed different patterns for formation of volatiles, which was interesting with regard to reaction pathways, and the GC–MS results correlated very well with some of the sensory data. Data from analysis of AnV and induction time were not relevant for assessment of the oil quality in this experiment.

TOH had no effect on oxidative deterioration of the odor and flavor of CLO, whereas TOH + AP had a positive impact with regard to formation of herring oil odor and flavors. However, other storage conditions such as free access to oxygen or lower temperature might lead to different effects. Only one concentration of each antioxidant was used, and considering that antioxidative effects are highly concentration-dependent, other levels of the antioxidants could give other results. AP has limited solubility in bulk oils, but use of ascorbyl oleate, which is less crystalline and more soluble (20), or combinations with substances such as rosemary extracts might be interesting.

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